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March 23, 1993

PHL63148.FI.PM
W.A. No. 18-3LY2.0

Mr. Harry Harbold, RPM
US EPA Region III
841 Chestnut St.
Philadelphia, PA 19107

Subject: Boarhead Farms Schedule for April/May 1993
Biota Investigation Scope of Work

CH2M HILL plans to be onsite at the Boarhead Farms site periodically for the next month (about once per week through April 12) to check on our field office in the absense of full-time security and full-time for approximately 2 weeks beginning April 12, 1993. Two primary activities will be occurring in April: completion of Phase 1 of the biota investigation (weeks of April 12 and April 19) and the second quarter sampling of residential wells (week of April 19). We plan to hook up utilities to the trailer the week of April 5. We intend to delay drilling until after the Removal Group has completed the Pond 11 test pit investigation; drilling is currently planned for start-up the week of May 10. Please inform Boarhead Corporation of our schedule.

I also want to bring to your attention to the attached memo from CH2M HILL's biota task leader regarding the remaining biota investigation. In February, you received from CH2M HILL a validation package for the sediment bioassay data (I have attached a copy of the data package to this letter for your convenience). One major concern described in the sediment bioassay data package is a high mortality rate in the control group that renders results of non-control groups questionable.

CH2M HILL has one more sediment bioassay sample to collect (Area 3) to complete the current scope of work. However, given the questionable results obtained from the initial round of samples, we feel that CH2M HILL and EPA biologists should revisit sediment bioassay sampling needs so that the

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April biota fieldwork can constitute the end of Phase 1. We have currently submitted a SAS request to CRL for between 1 to 9 sediment bioassay samples, should EPA decide that additional samples should be collected for bioassay in April. We need to resolve the number of samples by April 1. Please contact me as soon as possible with EPA's decision or to make arrangements for our biologists to discuss the issue.

Authorization to complete the biota task as described in Work Plan Addendum No. 2 is also needed. If additional bioassay samples are required, we will need to add a couple days of sampling to our cost estimate.

If you have any questions, please call me at 215-563-4220.

Sincerely,

CH2M HILL

Donna Connery

Donna S. Connery
Site Manager

Enclosures: Memo
Sed. Bioassay Data Package

cc: M. Tilchin, CH2M HILL/WDC

MEMORANDUM

CH2MHILL

TO: Donna Connery/PHL

COPIES: Koumudi Ketkar/WDC

FROM: Jim Stark

DATE: January 29, 1993

SUBJECT: Boarhead Farms Sediment Bioassay Data Validation

PROJECT: PHL63148.SA.DV

Enclosed with this memo is a table summarizing the results of the sediment bioassays using the amphipod (*Hyalella azteca*) and the midge (*Chironomus tentans*) on thirteen samples. The following are comments on these bioassays.

- On page 33 there is an error, stating that "both chronic tests were started on October 1, 1992". In fact the tests were started on four different dates (8/26/92, 9/3/92, 9/15/92,and 10/1/92) listed on page 31.

- The amphipod test started on 9/3/92 on Sample SC4354 did not have a control using organisms from the same lot or of the same age. The control organisms from the 8/26/92 test were used for data comparison with this (9/3) test. The use of different lot/age organisms as a control is inappropriate.

- On page 4 of the SAS, hand written comments specified that endpoints for organism weights be 0.001 mg and lengths be 0.01 mm. It is stated on page 33 that the organisms were weighed to the nearest 0.01 mg and amphipods measured to the nearest 0.1 mm. The values were reported to the requested levels (determined mathematically), but not measured to those levels.

- In the 8/26/92 amphipod test, three out of the four replicates in the control media had 0 percent survival. One replicate had 90 percent survival. Acceptable control survival is $\geq$ 80 percent survival. The contract laboratory considered these findings as an anomoly and suggested that the mortlities were due to contaminated glassware. If glassware is suspected of contamination, the vality of the test is questioned because the effects (mortality or reduced growth) observed in the sediment samples may have been influenced by potential contaminated glassware.

- In the midge tests conducted 9/15/92, the samples were stored 20 days longer than when they were tested using amphipods on 8/26/92. It is noted that in at least one of these samples (SC4354) the pH changed over time. In the earlier

amphipod test, pH was measured at approximately 12 and remained high (about 11) for the test duratioin. In the midge test, the pH started high (approximately 11) but continuously dropped during the test to about pH 8. This indicates that the chemistry of the sample had changed over time and that change may influence its toxicity to the test organisms.

- The raw data for the amphipod lengths (pages 41 - 49) were not identified well.

- The midge mean weight for Sample SC0776 was incorrect. The data analysis was on only three of the four replicate values. This, however, did not influence the test results.

- Midge survival calculations for three samples are incorrect: SC0770 (reported as 73.8%) should be 81.7%; SC0771 (reported as 85%) should be 63.8%; SC0776 (reported as 71.7%) should be 66.3%.

- No reference toxicant test data were included with the data package submitted. This data was requested in the SAS.

The following are general conclusions of the tests' results.

- Because the control amphipods in the 8/26/92 test did not meet acceptability criterion (≥80% survival) due to 3 of 4 replicates having complete mortality; the validity of the test results are questionable. To accept the results and interpret the observed effects in the sediment samples as indications of sediment toxicity, one must assume that the mortality in the controls was not due to unhealthy/stressed organisms or contaminated/poor dilution water quality or laboratory stress during handling. The assumption is that contaminated glassware caused the control mortality only and did not influence the sediment samples. That is, the glassware used for the test samples were not contaminated.

- No appropriate control was tested for the amphipod 9/3/92 test on Sample SC4354. Therefore, there is no means to confirm that the organisms and labware used in that test were acceptable. The use of a different control (organisms of a different lot and tested about one week earlier) is not acceptable. If the pH of Sample SC4354 is confirmed to be at the levels reported (>10), it is assumed that the mortality observed in this test was caused by the sediment sample and not an interference or artificial toxicity.

- Although the lengths and weights of organisms were not measured at the specified levels, they were mathematically converted to the proper level prior to statistical analysis.

The following is an interpretation of the bioassys results.

- Samples SC4349, SC4350, and SC4354 were chronically toxic to *Hyalella azteca* based on survival.
- Samples SC0770 and SC0774 were chronically toxic to *Hyalella azteca* based on length.
- Samples SC0770, SC0771, SC0772, SC0773, SC0774, SC0775, and SC0776 were chronically toxic to *Hyalella azteca* based on weight.
- Sample SC4354 was chronically toxic to *Chironomus tentans* based on survival.
- *Chironomus* survival in samples SC0771 (63.8%) and SC0775 (62.5%) may indicate toxicity if a different statistical analysis is used. For example, the point estimate method using the IC25 (concentration that is inhibited by 25 percent) is a more appropriate analysis for determining a biological effect.
- Although *Hyalella* survival in sample SC0774 (75%) was significantly different from the control survival (93.8%) it should not be considered toxic based on an IC25 point estimate.

BOARHEAD FARMS SEDIMENT BIOASSAYS SUMMARY OF RESULTS

		Hyalella azteca				Chironomus tentans	
Test Date	Sample No.	Mean % Survival	Mean Length (mm)	Mean Weight (mg)	Test Date	Mean % Survival	Mean Weight (mg)
8/26/92	CONTROL	90	4.39	0.195	9/15/92	91.3	0.617
8/26/92	SC4349	20*	4.31	0.199	9/15/92	Sample Lost	
8/26/92	SC4350	43.8*	5.28	0.227	9/15/92	88.8	2.135
8/26/92	SC4351	87.5	5.04	0.349	9/15/92	98.8	2.141
8/26/92	SC4352	95	5.03	0.296	9/15/92	83.8	1.848
8/26/92	SC4353	85	4.83	0.229	9/15/92	93.8	1.757
9/3/92	SC4354	0*	0*	0*	9/15/92	36.3*	0.345*
10/1/92	CONTROL	93.8	5.99	0.470	10/1/92	87.5	0.887
10/1/92	SC0770	86.3	4.28*	0.167*	10/1/92	81.7	1.474
10/1/92	SC0771	95	4.88*	0.240*	10/1/92	63.8	1.123
10/1/92	SC0772	77.5	4.83*	0.207*	10/1/92	82.5	1.949
10/1/92	SC0773	87.5	4.56*	0.168*	10/1/92	70	2.227
10/1/92	SC0774	75*	3.68*	0.095*	10/1/92	82.5	2.097
10/1/92	SC0775	100	5.29*	0.344*	10/1/92	62.5	2.124
10/1/92	SC0776	98.8	5.18*	0.236*	10/1/92	66.3	2.385

* Indicates significant difference from control data

CASE NARRATIVE

SAS ORDER NUMBER: 7429-C-01

NUMBER OF SAMPLES: 13

SAMPLE MATRIX: Soil

SAMPLE RECEIPT:

This report presents results of thirteen (13) sediment bioassays conducted using the freshwater amphipod, *Hyalella azteca*, and midge larvae, *Chironomus tentans*. The thirteen samples were received in six (6) shipments received between August 8 and September 25, 1992.

The following table summarizes dates for sample arrival and the start of individual bioassays.

SAMPLES NUMBER	SAMPLE IDENTIFICATION	SAMPLE RECEIPT	ASSAY START DATE	
			Amphipod Assay	Midge Assay
SC0770	Station CV2\2105	09/17/92	10/01/92	10/01/92
SC0771	Station VC-1\2106	09/17/92	10/01/92	10/01/92
SC0772	Station WT12-5\2109	09/19/92	10/01/92	10/01/92
SC0773	Station WT00-2\2110	09/19/92	10/01/92	10/01/92
SC0774	Station VC3-1\2111	09/25/92	10/01/92	10/01/92
SC0775	Station VC4-1\2112	09/25/92	10/01/92	10/01/92
SC0776	Station VC00-1\2113	09/25/92	10/01/92	10/01/92
SC4349	Station PD00-2/2100	08/14/92	08/26/92	S.L.
SC4350	Station PD8-1/2101	08/21/92	08/26/92	09/15/92
SC4351	Station PD11-2/2104	08/21/92	08/26/92	09/15/92
SC4352	Station PD11-10/2108	08/21/92	08/26/92	09/15/92
SC4353	Station PD10-2/2103	08/21/92	08/26/92	09/15/92
SC4354	Station PD9-3/2102	08/28/92	09/03/92	09/15/92

S.L. Insufficient sample to complete assay due to spill of sample container.

METHODS:

Bioassay methods used in this program are as follows:

U.S. EPA. 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Second Edition. EPA/600/4-89/001.

and

ASTM. 1990. *Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates*. Draft Document Prepared by Committee E-47. Draft Dated 05/01/90.

Summary of General Methods

Toxicological and analytical protocols used in this program follow procedures outlined in *Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA 1989), and *Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates* (ASTM 1990). These programs provide standard approaches for the evaluation of chronic toxicological effects on aquatic organisms.

Sediment samples provided by CH2M Hill between August 8 and September 24, 1992 were evaluated for chronic effects on survival and growth of *Hyalella azteca* and *Chironomus tentans*, for 28-day and 15-day exposure periods, respectively. Assays were conducted at the EnviroSystems, Inc. (ESI) laboratory in Hampton, New Hampshire.

Test Species

The amphipods, *Hyalella azteca*, were from stocks maintained by Aquatic Research Organisms (ARO) in Hampton, New Hampshire. Amphipods chosen for the test were between the second and third instar (2-3 mm in length) stages of development. The amphipods were cultured at test temperature and photoperiod. The amphipods were acclimated to the overlying water in the test chambers in the following manner: 2 hours in a 50:50 mixture of culture water and diluent, 2 hours in a 25:75 mixture of culture water and diluent, and then transferred to the test vessel.

The chironomids, *Chironomus tentans*, were also cultured at ARO. Animals selected were larvae in the second instar (10 days post hatch) stage of development. Chironomids were cultured under conditions similar to those used in the assay. During acclimation, temperatures were maintained at 20 $\pm$2°C and the photoperiod was 16/8 hours light/dark. Organisms were acclimated to synthetic moderately hard reconstituted test water over a one week period by replacing on a daily basis approximately 500 ml of the original culture water with an equal amount of the test diluent. Before adding the animals directly to the test vessels, they were further acclimated to the overlying water in the same manner as the amphipods.

Control Sediments and Dilution Water

Control sediment used in the amphipod tests was collected near the head of the Taylor River in Hampton Falls, New Hampshire. This stream is classified as A-1 by the State of New Hampshire. The stream is located in a rural area and is typified by low flow rates, sandy to muddy bottom, and moderate benthic vegetation in the form of grasses and other water plants. The stream ranges in width from less than ten feet to fifty feet with depths of less than one foot to six feet. The stream receives no industrial or municipal point source inputs. There are also no significant non point source inputs.

Control substrate used for the chironomid test was provided by ARO. This sediment has been used to successfully culture *C. tentans*.

Dilution water was synthetic moderately hard reconstituted laboratory water made according to EPA (1989).

Bioassay Technique

The chronic sediment test was conducted according to the methods of U.S. EPA (1989) and ASTM (1990). This protocol calls for the sifting of the test and control sediments to remove all large rocks, twigs, and other debris. Approximately 2 cm of the sediment were placed in the test vessel along with the moderately hard reconstituted laboratory water. Sediment and diluent were gently aerated overnight. Any floating detritus was removed from the water surface. The next day, the test-organisms were added below the surface of the test diluent. Both chronic tests were started on October 1, 1992.

The chronic amphipod test used four replicates with twenty animal per replicate for the control and test sediments. Test chambers were 1000 ml glass beakers with 2 cm of sediment and 750 ml of moderately hard reconstituted lab water. Each replicate was fed a 0.5 ml suspension of rabbit food and deionized water on Mondays, Wednesdays, and Fridays.

The chronic chironomid test used four replicates with twenty animals per replicate for each test treatment. Test chambers were 2000 ml glass beakers with 2 cm of sediment and 1500 ml of test diluent. Each replicate was fed a 0.5 ml suspension of Tetra-min® and cerophyll mix and deionized water on Mondays, Wednesdays, and Fridays.

Dissolved oxygen, specific conductance, pH, temperature, and survival were recorded on a daily basis. Alkalinity and hardness were measured in each replicate at the beginning and end of the test. Water levels were maintained by adding deionized water to the beakers. At the end of their respective exposure periods, sediments were placed on 0.5 mm screens and washed with water. Animals were transferred to 30 ml plastic cups, rinsed twice with deionized water, and preserved with alcohol. Final counts were recorded on the data sheets. Amphipods were photographed to measure lengths to the nearest 0.1 mm. All surviving animals from a replicate were placed on tared weigh pans, dried overnight at 60°C, and weighed to the nearest 0.01 mg.

Data Analysis

H. azetca survival, length, and weight data were analyzed using Dunnett's Test. *C. tentans* survival and weight data were analyzed using the Bonferroni T-Test and Wilcoxon Rank-Sum Test with a Bonferroni Adjustment (EPA 1989), respectively, to determine the statistical significance of any differences between the treatments and control. Replicate data were combined for this analysis. Statistical significance was accepted at $p < 0.05$.

RESULTS

Water quality data collected during the assays are summarized in the following tables. It should be noted that survival data reported at interim periods are estimates based on the number of dead organisms removed from the test chamber at any given period. In most cases the organisms were burrowed in the sediments with no indication of mortality evident during the assay.

In all cases the temperature of the overlying water in the test chambers was within the specified range of 20 ±2°C. The accuracy of the thermometers used in the assays allows measurement of temperatures are measured to 2 significant figures. Gentle aeration was applied to all test chambers during the assay. Dissolved oxygen levels remained above 60% saturation throughout the assay. pH levels varied with individual samples and over time.

Significant differences between survival and growth in the control and treatments are summarized in the following table. Cases where there was no observable statistical difference between the treatment and control are noted as "No Sign.", cases where a statistical difference between control and treatment existed are marked as "Sign".

SAMPLES NUMBER	SAMPLE IDENTIFICATION	AMPHIPOD ASSAY			MIDGE ASSAY	
		Surv.	Length	Weight	Survival	Weight
SC0770	Station CV2\2105	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0771	Station VC-1\2106	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0772	Station WT12-5\2109	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0773	Station WT00-2\2110	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0774	Station VC3-1\2111	Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0775	Station VC4-1\2112	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC0776	Station VC00-1\2113	Not Sign.	Sign.	Sign.	Not Sign.	Not Sign.
SC4349	Station PD00-2/2100	Sign.	Not Sign.	Not Sign.	S.L.	S.L.
SC4350	Station PD8-1/2101	Sign.	Not Sign.	Not Sign.	Not Sign.	Not Sign.
SC4351	Station PD11-2/2104	Not Sign.	Not Sign.	Not Sign.	Not Sign.	Not Sign.
SC4352	Station PD11-10/2108	Not Sign.	Not Sign.	Not Sign.	Not	Not
SC4353	Station PD10-2/2103	Not Sign.	Not Sign.	Not Sign.	Not Sign.	Not Sign.
SC4354	Station PD9-3/2102	Sign.	Sign.	Sign.	Sign.	Sign.

S.L. Sample Lost.

Of the two test species the amphipod appeared to be the more sensitive organism. Significant impacts on either growth or survival were noted in 10 of the 13 amphipod assays. Growth, measures as either weight or length, appeared to be a more sensitive indicator than did survival. Of the 10 cases exhibiting significant difference from the control 8 were observed to be related to growth factors, length and weight. Only one of the sediments, SC4354, resulted in significant impacts on the midge larvae. In this case, as with the amphipod there was no survival at the termination of the assay.

The amphipod assay for sample SC4354 was terminated on the 20th day of the assay. The assay was terminated early as there were no indications of any live amphipods in the sediments. The preliminary basis for termination of the assay prior to day 28 was the extremely high pH of the overlying water. Data from the first day of the assay showed pH levels ranging from 12.20 to 12.36 SU. The pH of the overlying water gradually decreased with time. At the end of the assay pH levels were still above 10.5 SU.

PROJECT PROBLEMS AND PROTOCOL DEVIATIONS

The following protocol deviations were noted during this study. During the first series of amphipod assays, started August 26, 1992, control survival in one replicate was 90% and 0% in the remaining three replicates. The cause for the abnormal survival in the three replicates could not be directly associated with any of the measured parameters; dissolved oxygen, pH or temperature. The health of the amphipods was also discounted as a potential source of the high mortality. This was based on the fact that survival in one of the control replicates was greater than the 80% minimum level (90% in replicate D) set for the assay. In addition overall amphipod survival in three of the six assays started at the same time exceeded the minimum 80% survival level. This data indicates that the test chambers may have been the source of the toxicity. It is possible that beakers were contaminated during the cleaning process. Based on these findings the low control survival observed in three of the four control replicates was considered to be anomalous. The data from the single control replicate, "D" was used to make further statistical comparisons.

The amphipod assay of sediment SC4354 was terminated on day 20 instead of day 28. The assay was terminated as there was no evidence of any living amphipods in the test chambers.

No data is available from the midge larvae assay for sediment SC4349. The sample container was dropped during sample preparation and the sediment spilled on the lab floor. The sample had a high water content which prevented it from being recovered from the floor.

The start of 5 of the midge larvae assays was beyond the specified sample holding time of 14 days. All amphipod assays were started within the specified holding time. Midge assays for samples SC4350, SC4351, SC4352 and SC4353 were started 24 days after sample receipt while the midge assay with sample SC4354 was started 17 days after receipt. It is not known what impact, if any the delay in starting the assay would have had on the outcome. The assay was delayed due to a lack of midge larvae of the proper age. The midge cultures maintained at ESI and its sister company ARO failed to produce sufficient numbers of eggs at the time of the start of the assay.

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, for other than the conditions detailed above. In addition, I certify that to the best of my knowledge and belief, the data as reported are true and accurate. Release of the data contained in this data package has been authorized by the LABORATORY Manager or his designee, as verified by the following signature.

LABORATORY MANAGER

Name	Title	Date
Kenneth A Lewis	President	12/22/92